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Introduction

Humans noticed inhibitory as well as promotory effects of certain plants since past hundreds of year. Some plants inhibit or promote other plants by means of chemicals they synthesize, a process known as 'Allelopathy'. Some may said that 'Allelopathy' as one of the parts of competition. To minimize confusion, term 'interference' may be used to encompass both 'Allelopathy' and 'competition' (Muller, 1969 Rice, 1984). Allelopathy **(Greek words: '*allelon*' means reciprocal and *'pathos'* means that happens to one)** is defined as "any direct or indirect harmful or useful effect by one plant on another through secretion of chemicals into the environment".

Since hundreds of years, farmers have been practicing crop rotation method to solve these problems developed due to Allelopathy of crop and weed plants. The "smother crops" like barley, rye, buckwheat, sweet clover, sunflower, Sudan grass and sorghum etc suppress the growth of weeds [Overland (1966) Rice (1979)].

Plants do not produce allelochemicals at all times. Some are present at all times while some are produced only after injuries or infection by pathogens. Their concentrations vary in different plant parts. Plants protect themselves against herbivores and pathogens by producing defence chemicals but may face problems of 'autotoxicity'. The allelochemicals released in the surrounding habitat may affect growth, anatomy or external morphology and physiological processes of other plants.

In agricultural land we have been facing problem of weed plants that compete with or influence main crops and reduce crop yield. This district is partly developed in upper Godavari as well partly in Bhima basin. The Ahmednagar district is the largest district of Maharashtra state. Newasa is one of 14 Tahasil of Ahmednagar district.

In agriculture point of view, the Newasa Tahasil is located in Shrirampur subdivision of district Ahmednagar in Maharashtra state, India. Geographically Newasa Tahasil is situated on 19^0 .37 North latitude and 75^0 East longitude. This tahasil includes 129 villages and also occupies about 1343.43 km^2 land in which around 1223.91km^2 land is subdivided into Agriculture, as well as 14.75km^2 land is acquired by the forest, and 104.77 km^2 space is used for the other purpose.

Agriculture in Newasa is mostly depending on southwest monsoon rains during the months of June to September, The monsoon is mostly irregular and insufficient. The average annual rainfall of Newasa tahasil is 550 to 600 mm. However, the rivers viz. Godavari and Pravara became the blessings to the farmers. The sufficient water for agriculture is made available through the facilities like Mula, Bhandardara dams & lift irrigation, back water from Jayakwadi dam (Nathsagar), with the support of these irrigation facilities, large area of this tahasil came under the cultivation. Soils of Newasa tahasil are deep black and fertile. Most of the farmers of Newasa tahasil prefer mono-cropping system. Imbalanced heavy doses of chemical fertilizers along with excessive irrigation, burning of agriculture waste, lack of organic fertilizers as well as no green manuaring resulted in making soil infertile. In some area of this tahasil the electrical conductivity of the soil is increasing and availability of organic carbon, macronutrient, and micronutrients are decreasing day by day. It resulted in damaging physical, chemical and biological properties of the soil. Due to unavailability of nutrients, crops suffer from deficiencies which ultimately resulted into the less crop production.

Amaranthaceae, a dicotyledonous family is abundant in tropical regions. It contains 174 genera and 2500 species, distributed worldwide. Plant species have scarious bracts, perianth and connate filaments. Flowers occur in dense inflorescence. Amaranthaceae family in Maharashtra state possesses 24 weeds, 2 medicinal plants,4 ornamental plants,2 medicinal species and 4 vegetables Almedo. It represented in Ahmednagar district with 8 genera, 12 species (Pradhan and Singh,1999).Plants may have allelopathic potentials. It should be remembered that different that different organs of a weed plant may have different allelopathic effects on same test plant. Amaranthaceae is characterized by its diversity in secondary metabolites, essential oils, sesquiterpenes, diterpenes, phenolic acids, flavonoids and betalains. Betalains are water soluble, natural pigments that occurs only 13 families of Order Caryophyllales. Phytochemical screening of available plant species will be done. Out of all plant species four to five species will be selected for the present study. Their allelopathic potentials will be found out.

So, the intention of present work is to formulate liquid or solid formulation that will benefit crop yield.

In India 78 % population depend on Agriculture. For getting higher agriculture yield most of the farmers have been using synthetic chemical fertilizer, pesticides and herbicides which resulted in causing yeild pollution. The control of weed is very serious problem among maharashtra farmers. Weeds are growing abundantly in yeild and causes

major loss in yield. The species of family Amaranthaceae have become serious weeds in the area of Newasa Tahasil of Ahmednagar district. Therefore this family is selected for present work whether these weeds promote or inhabit the growth of crops, will be studied.

Significance of Research Work

1.The Research will help to improve weed management Technique.

2. The Research will help to enhance organic produce of Crop.

3. The research will to improve physical, chemical and biological properties of Soil.

4.The research will help to study of Allelopathic effect of Amaranthaceae on selected test crops.

5. The research will help to understand Allelopathic chemicals of Amaranthaceae same species and their effect on crops plants.

6.The research will help to understand the antifungal and antibacterial properties of Amaranthaceae species.

7. The research will help to study the rhizosphere microflora of Amaranthaceae.

Study Area of Research Work

The district Ahmednagar lies Between 18.2to 19.2 ^{0}degree north latitude and 73.9 to 75.5 ^{0}eastern longitude, spread over 170448 km^2 This district includes 14 tahasilsnamely Newasa, Shevgaon, Pathardi, Parner, Rahata, Rahuri, Karjat, Jamkhed, Shrigonda, Kopargaon, Sangamner, Shrirampur, Ahmednagar,Akole. The District is subdivided into four subdivision namely, Shrirampur, Sangamner, Karjet and Ahmednagar.

The district is partially hillyand partially plain. Agriculture is main source of livelihood of the people Variety of Crops are grown in this district. Out of these wheat, gram, sugarcane, cotton, Bajra, Soyabean, onion are the major crops.

The Newasa tahasil is irrigated through Mula dam, Jayakwadi backwater and some part is irrigated through Bhandardara dam. the river Mula, Pravara and Godavari are the boon for this tahasil. But day by day yield has been decreasing due to the indiscriminate use of chemical fertilizers, pesticides and herbicides. The management of weed is becoming serious problem due to unavailability of labor. The use of synthetic chemicals caused soil pollution .The *Alternanthera* is very abundant weed .This weed is generally used as fodder for cattle in study area. Therefore the study of allelopathy of members of Amaranthaceae will help to understand whether weeds beneficial or harmful to the crops

Review of Literature

While studying the mechanism of plant interference both competition and allelopathy should be investigated. **Increase in concentration of allelopathic compounds is correlated with soil pH, soil moisture, soil nutrients and soil organic matter (** Inderjit and Moral ,1997).

Variable results were obtained while testing the effects of phenolic compounds on germination and seedling growth of weeds *viz.*, ***Chenopodium album****, Solanum nigrum,* ***Amaranthus retroflexus, Rumex crispus***. Allelochemicals are stimulatory or inhibitory at lower or higher concentrations respectively. **This work is in accordance with Pellissier's conclusion that allelopathic phenomena obey the all-or-none law.** The effect of a phenol is positive, null or negative, but never more or less toxic according to its concentrations (Gonzalez *et al.*,1997).

Control of a perennial, troublesome and aggressive weed 'cogon grass' (*Imperata brasiliensis* Trin. more is difficult due to its high reproductive capacity and rhizomes. Seed leachates of cover crops *viz. Pueraria phaseloides*, Roxb., *Canavalia ensiformis* L. and *Mucuna pruriens* var.*utilis* not only influenced germination but increased epicotyl growth and dry weight. Shoot extracts of these three plants promoted germination of test plant. However, dry weight was decreased by jackbean. Root extracts also had similar influence except promoting effect on dry weight. **Root exudates of** *Canavalia* **promoted N uptake but reduced P and K uptake while** *Mucuna pruriens* var.*utilis* **reduced NPK uptake.** Out of these three cover crops jackbean could be reliable for replacing Cogon grass (Cassini and Olivero ,2001).

Under aerobic conditions plant residues have less inhibition or stimulate growth than anaerobic conditions and their effects vary with time. Aeration affects phytotoxicity during **residue decomposition** (An *et al.* 2003).

Uptake of ^{32}P and ^{65}Zn was gradually decreased with the concentration of weed extracts. Inhibitory effect of the weeds is due to the presence of phenolics such as Gallic, Vanillic, Protocatechuic and p-hydroxybenzoic acids (Saxena *et al.*, 2003).

Weeds like ***Amaranthus gracilis*** **Desf**., *Convolvulus arvensis* L., *Lactuca serriola* L. and ***Portulaca oleracea*** L. possess allelopathic activities. Root exudates released by weeds when tested on crops *viz.*, cabbage, carrot, cucumber, onion, pepper, squash and tomato showed variable effects. **Soil-incorporated shoot residues** and decayed residues reduced seedling growth of test crops while surface-placed residues delayed seedling emergence (Obiad and Qasem, 2005).

Decrease in phytotoxicity was observed with increasing age of residues. The amount of N, P, K and micronutrients increased significantly in residue-amended soil. **As the residues become older all nutrients except N decreased.** *Parthenium* residues affect other crop plants by releasing phenolic rather than decreasing nutrients in the soil (Batish *et al.*, 2005).

Objectives

1. Survey of Study area.
2. Survey of Amaranthaceae family in study area.
3. *In-vitro* study of Allelopathy of some members of Amaranthaceae against some crops.
4. *In-vivo* study of Allelopathy of some members of Amaranthaceae against selected crops.
5. *In-vitro* study of Antifungal and Antibacterial activity of selected Amaranthaceae species.
6. Isolation of rhizosphere Microflora of Amaranthaceae.
7. Study of physiochemical properties of soil before and after crop plantation.
8. Study of promotory or inhibitory effect of Amaranthaceae against soil beneficial microflora.
9. Study of allelochemicals present in Amaranthaceae.

Research Methodology

1. Survey of Literature.

2. Survey will be done by visiting season wise crop fields. Samples will be collected; herbariums will be prepared and submitted to BSI Pune for identification and confirmation.

3. Various internet sites will be searched for data collection.

4. The Agronomy and Agriculture departments of Rahuri Krushi Vidhyapeeth will be visited to collect information about weeds. Plant materials were collected from agricultural lands of Tal Newasa. Fresh plant materials will be further used in laboratory bioassay.

Laboratory Bioassay: Extract/leachate analysis:

Separate extracts/leachates will be obtained by crushing different plant parts of selected weeds.10% stock solutions of each plant part in distilled water will be prepared, filtered with muslin cloth and Whatman filter paper No.1, stored in refrigerator and further diluted with distilled water to get extracts of different concentrations 10^{-1} to 10^{-3} (Narwal, 1994). Extracts were further used for bioassay in laboratory conditions. Test crop varieties selected for present studies:

Seeds of test crops will be procured from local Agricultural market.

Laboratory Bioassay: Effect of filtered fresh extracts/leachates of 10^{-1} to 10^{-3} concentrations on seedling growth parameters of test crop varieties will be recorded. Recording was done after 5th day. 0.01% Mercuric chloride was used to surface sterilize seeds followed by thorough washing with distilled water before use. Germination of seeds will be carried out in sterilized containers of 12cm diameter, using germination paper or Whatman No.1 filter paper. Triplicates of the containers will be maintained. 10 ml of extract was added in the Petri dishes/containers containing 10 seeds each. The slight emergence of radical will be considered as a sign of germination. Germination percentage will be calculated, Photographs will be taken. Percentage inhibition or stimulation of Shoot/plumule growth, Root/adventitious root growth and Total seedling growth, Germination over control will be calculated from which graphs will be drawn. Results obtained will be analyzed by single factor ANOVA variance (Multiple range test) followed by CD at 0.05% and Tukey's HSD test.

Estimation of proteins, Carbohydrates and chlorophylls in the leaves of Test Plants:

Biochemical analysis of leaves of test plants for proteins, chlorophylls and carbohydrates in treated and control plants will be done. Results will be tabulated and statistically analyzed. (Arnon, 1949 c. f. Sadasivam, 1997).

(B) Protein Estimation by Lowry's method (Lowry *et al*, 1951 c. f. Sadasivam, 1996):

(C) Estimation of Total Carbohydrates by Anthrone Method: (Hedge and Hofreiter 1962 c. f. Sadasivam 1996):

Qualitative Chemical analysis of plant materials

(A) Tests of Tannins and Phenols:

1. To 2ml aqueous extract of plant material add 5 % $Fecl_3$ solution=Deep blue black colour is obtained.
2. To 2ml aqueous extract of plant material add dilute iodine solution =Transient red colour iodine obtained
3. To 2ml aqueous extract of plant material add dilute HNO_3 solution =Reddish to yellow colour
4. To 2ml aqueous extract of plant material add dilute NH_4OH and potassium ferricynide solution = Red colour is obtained.
5. To 2ml aqueous extract of plant material add Acetic acid solution =Red colour obtained

(B) **Phytochemical Tests for Steroids (Salkowski Reaction):** To 2ml aqueous extract of plant material add 2ml chloroform and 2ml conc. H_2SO_4 = Chloroform layer appears red and acid layer greenish yellow fluorescence.

(C) **Phytochemical Tests for Organic acids:** Add NH_4OH to the aqueous extract of plant material .Add few drops of 5% $CaCl_2$ in 2ml test solution. White ppt. immediately is formed indicating presence of Oxalic acid. To Confirm this test add few drops of 5% lead acetate into 2 ml test solution resulting white ppt.

(D)Phytochemical Tests for alkaloids: Evaporate alcoholic/ aqueous or chloroform extract separately. To the residue add dil. HCL ,shake well and filter. Use filtrate for following test.

1. Dragendorffs test: 3ml filtrate + few drops of Dragendorff reagent = Orange brown ppt. is formed.

2. **Mayer's test:** 3ml filtrate + few drops of Mayer's reagent = ppt. is formed

3. Wagners test: 3ml filtrate + few drop of Wagner's Reagent = Reddish brown ppt.

(E) Phytochemical Tests for Flavonoids:

1. Add to a small quantity of plant residue lead acetate solution → Yellow ppt. is obtained.

2. Plant residue + NaOH in increasing amount → Residue shows yellow coloration which decolorizes after adding acid.

(F)Phytochemical Tests for Saponins:

1. To the extract of plant material add Chloroform, filter and a drop of conc. H_2SO_4 , 1ml acetic anhydride = blue / red/ blue/ green brown colour is obtained.

2. To the extract of plant material add DW and shake if foam is obtained indicating presence of Saponins.

(G) **Phytochemical tests for glycosides:**

Saponin glycoside: 10ml filtrate + warm side C_6H_6 → White ppt at Junction of C_6H_6 filtrate.

General glycosides: Plant extract filtrate+ Warm benzene by side → White ppt at junction.

(H) **Phytochemical tests for anthraquinone:**

Borntrager's test: 3ml extract + dil H_2SO_4. Boil and filter. Cool and add volume of benzene or Chloroform. Shake well. Separate organic solvent. Add ammonia./ NH_4OH Ammonical layer turns pink or red.

Modified Borntrager's test: 5ml extract + 5ml FeCl3 and 5 ml dil. HCl. Heat for 5minutes in boiling water bath. Cool and add benzene or any organic solvent. Shake well. Separate organic layer. Add equal vol. of dil. Ammonia.- Ammonical layer shows pinkish red co;our.

Analysis of plant materials for nitrogen, phosphorus and potassium:

In most of the methods organic compounds present in plant tissues are destructed thereby converting the elements to a soluble form for analysis (Huang and Shuttle, 1985; Linder and Harley, 1942; Parkinson and Allen 1985, c.f. Somwanshi *et al* 1980).

Isolation and Purification of nitrogen fixing Bacterial strains

The collected soil samples were used for the isolation of nitrogen fixing bacterial strains and their density on Jensen's nitrogen free Agar medium by serial dilution agar plate technique (Aneja 2002). Ten gram of soil from each sample was aseptically weighed and transferred to 250 ml conical flask containing 90 ml of sterile double distilled water. The flasks were kept on orbital shaker at 200 rpm for 30 minutes. Samples were allowed to settle for 1 minute. Aliquots of 1 ml of the supernatant from the sample were transferred to 9 ml of (0.85 % NaCl) saline solution in test tube and serially diluted up to 10^{-7}. 2 ml of sample from each of these dilutions was transferred to sterilized 18 ml of Jensen's agar medium before solidification (45^0 c temperature) and poured in sterile Petri dish. After solidification plates were kept in an incubator at 28^0 C (± 2) for 4- 5 days. After incubation microbial colonies were grown, these colonies count was measured on colony counter and further purified by transferring on Jensen agar medium by plate streaking. To avoid contamination flucanozole tablets were used in medium. This procedure was repeated until pure culture colonies were obtained. The pure culture colony thus isolated were tested for grams staining, pigmentation formation, colony characteristics, then kept in refrigerator after inoculating on slants of Jensen agar and used for further studies.

Evaluation of Bacterial strains by Nitrogen fixing ability Test -

The isolates were inoculated on Jensen and Ashby's nitrogen free Manitol and incubated at 30^0 C for 48 hours. Those isolates have showed better bacterial growth, were considered as nitrogen fixing strains. Simultaneously the Jensen broths were prepared by using bromothymol blue indicator and sterilized. The cultures of various isolates were prepared in Jensen's medium and used for inoculation in Jensen broth containing bromothymol blue. After inoculation these broths were kept on rotary shaker at 121 RPM for seven days. The color of medium was

recorded. The blue color formation represents highest nitrogen fixing ability of the strains. The blue color forming strains were selected for further studies.

Analysis of nitrogen fixing capacity of Bacterial isolate and Screening

The pure culture medium was prepared by using Jensen nitrogen free media composition. Bromothymol blue indicator was used. The nitrogen fixing strains were identified on the basis of color. The green color turns into blue indicate that, nitrogen fixing strains are developed in broth culture.

One colony of the culture was inoculated on another freshly prepared medium. This culture was used to test nitrogen fixation bacteria in broth. For this 50 ml aliquots of broth taken in 250 ml conical flask for inoculation. This broth was kept 12 days on rotary shaker at 121 rpm at 28°C. The purity of culture was tested by streaking the broth culture on plate. The broth cultures were kept on water bath at 50 to 60 ^{0} C temperatures to dry, and then dry culture was washed and used as sample. Simultaneously same procedure was followed to the broth of Jensen medium without inoculation as control.

Total nitrogen fixation by the Azotobacter isolates in the growing medium is quantified by the method (Johan Kjeldahl 1883). The amount nitrogen fixed per gm glucose consumption was calculated.

Nitrogen fixing capacity was tested of all strains. Out of 100 strains isolated from rhizosphere soil, only 25 strains five best strains of each monocot were selected based on their maximum nitrogen fixing capacity for seedling germination test.

Seed germination bioassay

The seeds will be procured from Krushi Seva Kendra. The seeds will be dipped in water those seeds settles at bottom that seeds will be selected for surface sterilization. The seeds will be surface sterilized with 95% ethanol (30 sec.) and again 0.1% $HgCl_2$ (5 Min.). After that, seeds will be washed 5 times with sterilized distilled water (Paul *et. al.* 2002). These seeds will be soaked in the extracts of selected plant material separately. The petri plates and filter papers were sterilized and the plate containing filter paper will be moistened with sterilized distilled water and 5 seeds will be placed in each plate for incubation for six days.

Soil analysis

1.Determination of available nitrogen in the soil. Alkaline $KMno_4$ method, (Subbiah and Asija, 1956 Somawanshi, *et al* 2012)

2. Determination of available Phosphorus in soil Olsen's method or sodium bicarbonate extractable 'P': (Olsen, et al 1954, c. f. Patil, 2007)

3.Determination of available Potassium in soil: (Knudsen and Peterson, 1982, Nazirkar 2007)

Antifungal and Antibacterial activity:

- **Collection of plant materials:** Plant material will be collected from Newasa Tehsil, District Ahmednagar, Maharashtra, India.
- **Preparation of PDA media and inoculation of fungal species:** PDA media will be prepared by standard methods. Six wells will be prepared in per petri dish (Three for control and Three for treatment). The culture of *Aspergillus niger, Aspergillus flaves, Penicillium chrysogenum and Paecilomyces lilacinus* will be used.
- **Preparation of Plant Extract (Concentrated Broth):** Plant material will be washed by distilled water two to three times, after washing the plant except roots will be used for preparation of plant extract. 100gm plant material will be crushed into the sterile distilled water in mortar and pestle and filtered by muslin cloth. After filtration the 50gm of plant extract will be dissolved into the 50gm of sterile distilled water (w/w), this 100gm of diluted extract will be filter twice to removes the debris in it. The liquid extract will be boiled at 50^0C up to one hour.
- **Treatment:** The two drops of concentrated plant extract will be added into the PDA media of three well of each plate as a treated and into remaining three wells sterile distilled water was added as a positive control. This media were incubated in incubator at 30^0C for 15 days. After 15 days the photographs and radial growth will be measure in millimetre (mm).

- SCOPE: Weeds are not always harmful to the crops. These either promote or inhibit growth of crops. There is in general differential effect of weeds on genetically different crop varieties or crops give differential response to the weeds. Weeds which promote crop development and yield should be investigated and incorporated in agricultural system.
- LIMITATIONS: Present study focuses mainly on finding out allelopathic potentials of wild, aggressive, naturalized common weed. The study will be limited to find out effect of on some morphological and reproductive growth parameters, yield of the selected test crop plants. Chemical analysis of weeds *(*HPTLC analysis) for finding out

allelochemicals responsible for inhibition or promotion of growth of test crop plants will be done in further advanced research.

- VALUE OF RESEARCH: The present research is important for the following reasons: Finding out allelopathic potentials of a wild and naturalized common weeds (Useful) and their uses in growing weed-free (troublesome), disease-free and healthy agricultural crops without using harmful toxic synthetic biocides.

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